Journal of Cellular Biochemistry 88:24–28 (2003)

PTEN Regulation of Neural Development and CNS Stem Cells

Li Li, Fenghua Liu, and Alonzo H. Ross*

Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, Massachusetts 01605

Abstract Even though phosphorylation of phosphatidylinositols by phosphoinositide 3-kinase (PI3K) has an important and pervasive role in the nervous system, there is little known about the phosphatases that reverse this reaction. Such a phosphatase, phosphatase and tensin homologue deleted on chromosome 10 (PTEN), was cloned as a tumor suppressor for gliomas. PTEN is expressed in most, if not all, neurons and is localized in the nucleus and cytoplasm. Recently, a series of papers using PTEN conditional knockouts has greatly extended our knowledge of PTEN's role during development. Loss of PTEN results in disorganization of the brain, probably due to a flaw in cell migration. In addition, there is a gradual increase in the size of neuronal soma, mimicking Lhermitte-Duclos disease. Recent experiments in our laboratory with adult PTEN +/− mice demonstrate that PTEN regulates migration of precursor cells in the subventricular zone to the olfactory bulb. We also found that PTEN haploinsufficiency can protect precursor cells from apoptosis in response to oxidative stress. Collectively, these studies demonstrate that PTEN does much more than suppressing tumors. It is a master regulator in developing and adult brain. J. Cell. Biochem. 88: 24–28, 2003.

Key words: phosphatase; phosphatidylinositol; CNS stem cells; tumor suppressor; development; glioma

Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) was originally cloned as a tumor suppressor for gliomas [Li et al., 1997; Steck et al., 1997]. We now know that PTEN is deleted or inactivated in many tumor types, including endometrial, melanoma, and prostrate, identifying PTEN as an important tumor suppressor. In addition, PTEN germline mutations can result in Cowden disease, Bannayan-Zonana syndrome, and Lhermitte-Duclos disease, in which disorganized hamartomas appear in multiple organs. Some of the patients with these disorders also show defects in neural development, including macrocephaly, mental retardation, cerebellar hypertrophy, ataxia, and seizures. As a result of its clinical relevance, PTEN is now a subject of intense study in many laboratories.

The PTEN protein is a phosphatidylinositol phosphate (PIP) phosphatase [Maehama and Dixon, 1998] specific for the 3-position of the inositol ring. Although, PTEN can dephosphorylate PI(3)P, PI(3,4)P_2, or PI(3,4,5)P_3 (PIP_3), it is likely that PIP_3 is the most important substrate in vivo. PTEN and phosphoinosotide 3-kinase (PI3K) have opposing effects on PIP_3 levels (Fig. 1). By lowering PIP_3 levels, PTEN decreases Akt activity and, thereby, enhances the rate of apoptosis [Datta et al., 1999]. PTEN decreases cell motility via small G proteins [Liliental et al., 2000] and may also inhibit cell migration by dephosphorylating focal adhesion kinase (FAK) [Tamura et al., 1998].

The biological functions of PTEN have also been analyzed by genetic methods. For *C. elegans*, PTEN and PI3K have opposing effects on worm longevity, dauer formation, and brood size [Ogg and Ruvkun, 1998; Gil et al., 1999; Mihaylova et al., 1999; Rouault et al., 1999]. PTEN and PI3K play opposing roles in regulating *Drosophila* development and cell size [Goberdhan et al., 1999; Huang et al., 1999]. Furthermore, the phenotype induced by PTEN mutation can be reversed by a mutation in the

Grant sponsor: NIH; Grant numbers: NS21716, CA68426, NS43879.

*Correspondence to: Alonzo H. Ross, Department of Biochemistry and Molecular Pharmacology, 364 Plantation St., Rm. 819, Worcester, MA 01605.
E-mail: Alonzo.Ross@umassmed.edu.

Received 31 July 2002; Accepted 5 August 2002
DOI 10.1002/jcb.10312

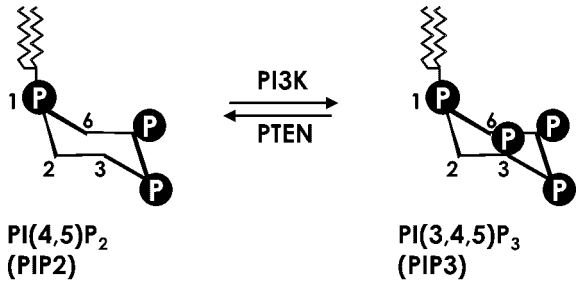

Fig. 1. PTEN dephosphorylates PIP3, thereby, reversing the reaction catalyzed by PI3K.

downstream kinase, Akt [Stocker et al., 2002]. Hence, PIP_3 is the critical substrate for *Drosophila* PTEN, and Akt is the crucial downstream regulator.

Knockout mice lacking PTEN are embryonic lethal [Di Cristofano et al., 1998; Stambolic et al., 1998; Suzuki et al., 1998]. PTEN loss leads to poorly organized ectodermal and mesodermal layers and overgrowth of the cephalic and caudal regions. PTEN +/− mice are viable but have an autoimmune disorder, diminished Fas-mediated apoptosis, and a high incidence of cancer [Di Cristofano et al., 1999]. These studies demonstrate the quantitative requirement for PTEN phosphatase and verify the identification of PTEN as a tumor suppressor. However, they offer no insight into the role of PTEN in the nervous system.

EXPRESSION OF PTEN IN THE NERVOUS SYSTEM

Since PTEN is a tumor suppressor for brain tumors, a natural question is which cells in the brain express PTEN? The first report of PTEN in the brain was based on examination of human biopsy samples [Sano et al., 1999]. There was strong PTEN immunostaining for neuronal soma and, particularly, nuclei. In addition, PTEN mRNA and protein was detected in cultures of cerebellar granule neurons [Kyrylenko et al., 1999].

We examined PTEN expression in adult mouse brain and detected PTEN-positive neurons in many sites, including mitral, periglomerular, and granule neurons in the olfactory bulb, pyramidal neurons in the cortex, magnocellular neurons in the basal forebrain, hippocampal and amygdalar neurons, and cerebellar Purkinje and granule neurons [Lachyankar et al., 2000]. Staining was most evident for large neurons and appeared to be confined to cell bodies. We did not detect staining of neuronal processes or glial cells.

We also assessed PTEN expression in two neuronal differentiation models. For pheochromocytoma PC12 cells, treatment with nerve growth factor (NGF) induces neuronal differentiation and expression of PTEN [Lachyankar et al., 2000]. For precursor cells from the subventricular zone (SVZ), we also observed induction of PTEN during neuronal differentiation. In contrast, we noted a weak, transient expression of PTEN in SVZ precursor cells differentiating to astrocytes. Suppression of PTEN expression with antisense oligonucleotides decreased neuronal differentiation for both PC12 cells and SVZ precursor cells but did not inhibit differentiation of SVZ precursor cells to astrocytes. These results demonstrate a requirement for PTEN during neuronal differentiation.

We examined the subcellular localization of PTEN for differentiated PC12 cells and SVZ precursor cells [Lachyankar et al., 2000]. By immunofluorescence microscopy and subcellular fractionation, PTEN is both nuclear and cytoplasmic. Also, PC12 cells expressing recombinant PTEN showed nuclear localization. These results are in good agreement with the original study reporting PTEN in the brain [Sano et al., 1999].

ANALYSIS OF PTEN WITH CONDITIONAL KNOCKOUT MICE

Recently, a number of groups have prepared PTEN conditional knockout mice utilizing the Cre-lox system. In the first of these studies, PTEN-lox mice were mated with mice bearing a nestin promoter-driven Cre transgene, thereby, deleting PTEN in neural precursor cells [Groszer et al., 2001]. Loss of PTEN from neural precursor cells had profound effects on the developing brain. The resulting mice were born with open eyes and enlarged brains and died shortly after birth. The laminar patterning of the cortex, hippocampus, and cerebellum was disorganized, likely due to a substantial flaw in cell migration (Table I). These authors also observed increased proliferation in the ventricular zone and decreased apoptosis. Total brain cultures were analyzed by flow cytometry, revealing that loss of PTEN resulted in an increase in cell size.

Two research groups mated PTEN lox mice with GFAP promoter-driven Cre mice. Both

TABLE I. Effects of PTEN Deletion Using Lox Mice

Cre promoter and references	Cell type undergoing PTEN deletion	Effect on tissue organization	Effect on cell proliferation	Effect on apoptosis	Effect on cell size	Effect on brain size	Mouse phenotype
Nestin [Groszer et al., 2001]	Neural precursor cells during development	Disorganization of cortex, hippocampus, and cerebellum	Increased BrdU labeling of ventricular zone	Decreased TUNEL staining of ventricular zone	Increased	Increased	Mice born with open eyes and die shortly after birth
GFAP [Backman et al., 2001]	Neurons	Disorganization in cerebellum and dentate gyrus	BrdU labeling unchanged in external granule layer	TUNEL staining unchanged in external granule layer	Neuronal soma increased but not other cell types	Increased, particularly cerebellum	Seizures and ataxia followed by death by 29 weeks
GFAP [Kwon et al., 2001]	Neurons, primarily postnatal	Disorganization of cerebellum	No change in Ki67 labeling in external granule layer	No change in ISEL staining in external granule layer	Neuronal soma increased	Increased, particularly cerebellum	Seizures and ataxia, death by 48 weeks
En2 [Marino et al., 2002]	Neurons and glia at midbrain hindbrain junction, about embryonic day 9.5	Disorganization of cerebellum and loss of foliation	BrdU and phospho histone H3 staining decreased	Decreased TUNEL staining	Increased precursor cell size	Increased, particularly cerebellum	Ataxia, degeneration of Purkinje cells
L7 [Marino et al., 2002]	Purkinje cells, primarily postnatal	Slight irregularities in Purkinje cell organization	Not determined	Not determined	Increased	None	Slow degeneration of Purkinje cells

groups expected to see PTEN deletion in astrocytes but instead noted deletion of PTEN in many neurons, occurring primarily postnatally [Backman et al., 2001; Kwon et al., 2001]. The resulting mice developed seizures and ataxia and died by 29–48 weeks. There was disorganization of the cerebellum and dentate gyrus. The loss of PTEN had no apparent effect on proliferation or apoptosis, but there was both an increase in neuronal soma size and overall brain mass. The increase in soma size was unique to neurons. Embryonic stem cells, embryonic fibroblasts, and thymocytes lacking PTEN did not show increased size [Backman et al., 2001]. In addition, these mice may provide a model for Lhermitte-Duclos disease in which there also is an increase in the size of neuronal cell bodies. Finally, these authors also noted that for normal mice, PTEN expression is much greater for neurons than glia [Backman et al., 2001].

Using an En2 promoter-driven Cre mouse, PTEN was deleted at embryonic day 9.5 in both neurons and glia at the midbrain-hindbrain junction [Marino et al., 2002]. These mice displayed ataxia and reduced activity. There was degeneration of Purkinje cells with progressive vacuolation and cytoplasmic accumulation of neurofilaments. There also was decreased cell proliferation in the cerebellum.

The effect of PTEN loss on apoptosis was complex. At E15.5, there was decreased apoptosis in the cerebellum, but at P1, apoptosis was unchanged by PTEN deletion. The authors proposed that the decrease in apoptosis was more influential, ultimately resulting in an enlarged cerebellum.

Finally, using an L7 promoter, PTEN was deleted postnatally in Purkinje cells [Marino et al., 2002]. There were small irregularities in Purkinje cell organization, and the Purkinje cells showed an increase in size and thickening of dendrites and axons. Eventually, the Purkinje cells showed degeneration and cell loss. Because in this experiment, the deletion of PTEN is so limited, the effects on Purkinje cells are undoubtedly cell autonomous.

ROLE OF PTEN IN ADULT NEUROGENESIS

The subventricular zone is the site of greatest proliferation in the adult brain [Peretto et al., 1999]. The resulting precursor cells migrate along the rostral migratory stream to the olfactory bulb. There they migrate radially and

form new neurons in the outer layers of the olfactory bulb. A critical step in analyzing the SVZ precursor cells was the development of a cell culture system [Reynolds et al., 1992]. These cells grow as large aggregates known as neurospheres and can differentiate to neurons, astrocytes, and oligodendrocytes. An intriguing question is how the SVZ finds the correct balance between proliferation, apoptosis, and migration and, thereby, sustains the correct number of SVZ stem/precursor cells for the life of the organism.

To analyze the possible role of PTEN, we carried out a comparison of SVZ neurosphere cells from PTEN +/+ and +/− mice [Li et al., 2002]. Even though PTEN levels in the PTEN +/− neurospheres were about 75% of those for PTEN +/+ neurospheres, phospho-Akt levels were increased by 2.4-fold (Fig. 2). We then tested the physiological consequences. Haploinsufficiency for PTEN had only minor effects on the rate of cell proliferation. Motility and migration were measured by filter penetration assays. PTEN +/− precursor cells showed greater motility and invasiveness than PTEN +/+ cells. Finally the role of PTEN in oxidative stress-induced apoptosis was assessed. SVZ precursor cells were treated with H_2O_2 and then were analyzed by TUNEL staining. Neurosphere cells from PTEN +/− mice were resistant to apoptosis. Hence, for PTEN +/− precursor cells, the small decrease in PTEN levels affects motility, invasiveness and apoptosis but not proliferation (Fig. 2).

We next analyzed the SVZ precursor cells in vivo. PTEN +/+ and +/− mice showed no difference in the rate of cell proliferation [Li et al., 2002]. However, migration of the SVZ cells to the olfactory bulb was more rapid for PTEN +/− cells than for +/+ cells. We also observed decreased numbers of apoptotic cells in the SVZ. However, the olfactory bulbs for PTEN +/− mice were of the same size as +/+ mice. The most likely explanation is that the rapid exit of PTEN +/− cells from the SVZ decreases the number of TUNEL+ cells, and the resulting surplus of cells is reduced by apoptosis in the rostral migratory stream or olfactory bulb.

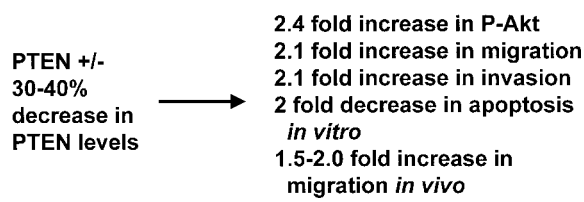

Fig. 2. Small changes in PTEN levels significantly affect the biological properties of SVZ precursor cells [Li et al., 2002].

CONCLUSIONS AND QUESTIONS

These studies establish a number of clear conclusions. First, PTEN is particularly influential in the regulation of cell motility and migration. This effect is observed even in PTEN +/− mice. Second, neuronal soma for PTEN −/− mice are enlarged. This effect is apparently unique to neurons and not observed for other cell types. Third, these studies help us to understand why PTEN is such a potent tumor suppressor. Cells have trouble compensating for even small decreases in PTEN levels. There are other PIP phosphatases expressed in the brain, and one would expect that they could compensate for loss of one copy of PTEN. However, for unknown reasons, they do not.

There are two points that still need to be resolved. First deletion of PTEN has substantially different effects on cell proliferation and apoptosis depending on which promoter is used to drive Cre expression (Table I). Our view is that these apparent disagreements indicate that PTEN regulation varies depending on cell type and stage of development. However, it is also possible that the altered migration for PTEN −/− cells causes cells to be misplaced and overlooked during counting. A second question is the role of PTEN in lineage determination. We reported that suppression of PTEN expression with antisense oligonucleotides inhibited neuronal differentiation for SVZ precursor cells [Lachyankar et al., 2000]. The cells differentiating to neurons instead underwent apoptosis. However, none of the studies with PTEN lox mice suggested a role in lineage determination (Table I). Recently, we repeated these experiments using PTEN −/− SVZ precursor cells and obtained similar results (Li et al., data not shown). Hence, our current working hypothesis is that SVZ precursor cells may be unique in this requirement. PTEN may be part of the regulatory circuits that maintain stem/precursor cells in the SVZ, migrating neuronal precursors in the rostral migratory stream and olfactory neurons at proper levels for the life of the organism.

REFERENCES

Backman SA, Stambolic V, Suzuki A, Haight J, Elia A, Pretorius J, Tsao MS, Shannon P, Bolon B, Ivy GO, Mak TW. 2001. Deletion of Pten in mouse brain causes seizures, ataxia and defects in soma size resembling Lhermitte-Duclos disease. Nat Genet 29:396–403.

Datta SR, Brunet A, Greenberg ME. 1999. Cellular survival: A play in three Akts. Genes Dev 13:2905–2927.

Di Cristofano A, Kotsi P, Peng YF, Cordon-Cardo C, Elkon KB, Pandolfi PP. 1999. Impaired Fas response and autoimmunity in PTEN+/– mice. Science 285:2122–2125.

Di Cristofano A, Pesce B, Cordon-Cardo C, Pandolfi PP. 1998. Pten is essential for embryonic development and tumour suppression. Nat Genet 19:348–355.

Gil EB, Link EM, Liu LX, Johnson CD, Lees JA. 1999. Regulation of the insulin-like developmental pathway of Caenorhabditis elegans by a homolog of the PTEN tumor suppressor gene. Proc Natl Acad Sci USA 96:2925–2930.

Goberdhan DCI, Paricio N, Goodman EC, Mlodzik M, Wilson C. 1999. Drosophila tumor suppressor PTEN controls cell size and number by antagonizing the Chico/PI3-kinase signaling pathway. Genes Dev 13:3244–3258.

Groszer M, Erickson R, Scripture-Adams DD, Lesche R, Trumpp A, Zack JA, Kornblum HI, Liu X, Wu H. 2001. Negative regulation of neural stem/progenitor cell proliferation by the Pten tumor suppressor gene in vivo. Science 294:2186–2189.

Huang H, Potter CJ, Tao W, Li D-M, Brogiolo W, Hafen E, Sun H, Xu T. 1999. PTEN affects cell size, cell proliferation and apoptosis during Drosophila eye development. Development 126:5365–5372.

Kwon CH, Zhu X, Zhang J, Knoop LL, Tharp R, Smeyne RJ, Eberhart CG, Burger PC, Baker SJ. 2001. Pten regulates neuronal soma size: A mouse model of Lhermitte-Duclos disease. Nat Genet 29:404–411.

Kyrylenko S, Roschier M, Korhonen P, Salminen A. 1999. Regulation of PTEN expression in neuronal apoptosis. Mol Brain Res 73:198–202.

Lachyankar MB, Sultana N, Schonhoff CM, Mitra P, Poluha W, Lambert S, Quesenberry PJ, Litofsky NS, Recht LD, Nabi R, Miller SJ, Ohta S, Neel BG, Ross AH. 2000. A role for nuclear PTEN in neuronal differentiation. J Neurosci 20:1404–1413.

Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliaresis C, Rodgers L, McCombie R, Bigner SH, Giovanella BC, Ittmann M, Tycko B, Hibshoosh H, Wigler MH, Parsons R. 1997. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 275:1943–1947.

Li L, Liu F, Salmonsen RA, Turner TK, Litofsky NS, Di Cristofano A, Pandolfi PP, Jones SN, Recht LD, Ross AH. 2002. PTEN in neural precursor cells: Regulation of migration, apoptosis, and proliferation. Mol Cell Neurosci 20:21–29.

Liliental J, Moon SY, Lesche R, Mamillapalli R, Li D, Zheng Y, Sun H, Wu H. 2000. Genetic deletion of the Pten tumor suppressor gene promotes cell motility by activation of Rac1 and Cdc42 GTPases. Curr Biol 10:401–404.

Maehama T, Dixon JE. 1998. The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-triphosphate. J Biol Chem 273:13375–13378.

Marino S, Krimpenfort P, Leung C, Van Der Korput HA, Trapman J, Camenisch I, Berns A, Brandner S. 2002. PTEN is essential for cell migration but not for fate determination and tumourigenesis in the cerebellum. Development 129:3513–3522.

Mihaylova VT, Borland CZ, Manjarrez L, Stern MJ, Sun H. 1999. The PTEN tumor suppressor homolog in Caenorhabditis elegans regulates longevity and dauer formation in an insulin receptor-like signaling pathway. Proc Natl Acad Sci 96:7427–7432.

Ogg S, Ruvkun G. 1998. The C. elegans PTEN homolog, DAF-18, acts in the insulin receptor-like metabolic signaling pathway. Mol Cell 2:887–893.

Peretto P, Merighi A, Fasolo A, Bonfanti L. 1999. The subependymal layer in rodents: A site of structural plasticity and cell migration in the adult mammalian brain. Brain Res Bull 49:221–243.

Reynolds BA, Tetzlaff W, Weiss S. 1992. A multipotent EGF-responsive striatal embryonic progenitor cell produces neurons and astrocytes. J Neurosci 12:4565–4574.

Rouault J-P, Kuwabara PE, Sinilnikova OM, Duret L, Thierry-Meg D, Billaud M. 1999. Regulation of dauer larva development in Caenorhabditis elegans by daf-18, a homologue of the tumour suppressor PTEN. Curr Biol 9:329–332.

Sano T, Lin H, Chen X, Langford LA, Koul D, Bondy ML, Hess KR, Myers JN, Hong YK, Yung WK, Steck PA. 1999. Differential expression of MMAC/PTEN in glioblastoma multiforme: Relationship to localization and prognosis. Cancer Res 59:1820–1824.

Stambolic V, Suzuki A, Lois de la Pompa J, Brothers GM, Mirtsos C, Sasaki T, Ruland J, Penninger JM, Siderovski DP, Mak TW. 1998. Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. Cell 95:29–39.

Steck PA, Pershouse MA, Jasser SA, Yung WKA, Lin H, Ligon AH, Langford LA, Baumgard ML, Hattier T, Davis T, Frye C, Hu R, Swedlund B, Teng DHF, Tavtigian SV. 1997. Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat Genet 15:356–362.

Stocker H, Andjelkovic M, Oldham S, Laffargue M, Wymann MP, Hemmings BA, Hafen E. 2002. Living with Lethal PIP3 levels: Viability of flies lacking PTEN restored by a PH domain mutation in Akt/PKB. Science 295:2088–2091.

Suzuki A, de la Pompa JL, Stambolic V, Elia AJ, Sasaki T, del Barco Barrantes I, Ho A, Wakeham A, Itie A, Khoo W, Fukumoto M, Mak TW. 1998. High cancer susceptibility and embryonic lethality associated with mutation of the PTEN tumor suppressor gene in mice. Curr Biol 8:1169–1178.

Tamura M, Gu J, Matsumoto K, Aota S-i, Parsons R, Yamada KM. 1998. Inhibition of cell migration, spreading, and focal adhesions by tumor suppressor PTEN. Science 280:1614–1617.